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Remarks

The Applicants hereby set out their reasoned explanation as to their proposed traversing of the Examiner's objection to, and rejection of, claims 1-32, as contained in the Official Action mailed on January 15, 2003.

PRIORITY

As clarified by the Examiner by telephone conversation at 8:50 AM MST, July 8, 2003; the Examiner found no support for the limitations contained in Claim 6 in the priority document, United Kingdom Provisional Application 9421223.0 filed October 20, 1994. Furthermore, the Examiner found no support for the limitations contained in Claim 8 in the priority document, United Kingdom Provisional Application 9421223.0 filed October 20, 1994. These allegations are respectfully traversed for the reasons now following.

Turning first to Claim 6, wherein the examiner states that there is no support within the above-noted priority document for the limitation of a "foreign gene is selected from eukaryotic or prokaryotic cells". In this regard, Applicants submit that the priority document, together with the known art at the time filing, fully supports the limitation in claim 6. The present invention is, *inter alia*, directed towards a method for detecting the expression of a transferred foreign gene in the tissues of a mammalian subject having undergone gene therapy. It was well known in the art at the time of filing of the priority document that a wide variety of viral, prokaryotic and eukaryotic genes had been suggested for use in gene therapy. It was also known that viral,

prokaryotic and eukaryotic genes appropriately promoted and enhanced for use in an eukaryotic system may be functionally expressed in the eukaryotic system (see for example Williamson et al., Appl. Environ. Microbiol. (1994) 60(3): 771-776).

Thus, the Applicants, as persons skilled in the art, were well aware of such references as Williamson et al cited above, and were accordingly well aware at the time of filing of the priority document that a variety of foreign genes could be used in the present invention. It is submitted a person skilled in the art would have no trouble understanding the invention extends to use of not only foreign genes selected from viral cells, but also foreign genes selected from eucaryotic or prokaryotic cells.

Moreover, and contrary to the examiner's suggestion, the priority document makes express mention that eukaryotic genes may be functionally expressed. For example, on p.1, lines 30-37, Applicant states that the use of the gene for viral thymidylate synthase (TS), viral polymerase and eukaryotic cytosine deaminase are examples of genes used in gene therapy. Further, on p.3, lines 11-12, Applicant states that "other viral genes or other chemosensitizing genes [of which eukaaryotic and prokaryotic genes are a subset] employed for gene therapy" can be used in the present invention.

Therefore, it is respectfully submitted that based on the priority document and the state of the art at the date of filing of the priority document there is sufficient support for claim 6, namely, that the selected gene can be from a viral, prokaryotic or eukaryotic source. It would have been reasonably predictable that a foreign gene from a prokaryotic or eukaryotic source could be transferred into a mammalian cell and that the detection of the expression of such a gene could be accomplished by the method as claimed in claim 1.

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With respect to claim 8, the examiner found no support for the limitation of a foreign gene being selected from human cytomegalovirus, varicella zoster, or Epstein-Barr virus, as recited in claim 8 of the current application. Applicants respectfully submit that the priority document clearly discloses that other viral genes (other than HSV-TK) could be used. In particular, on p.3, lines 8-10, Applicants state "the diagnostic imaging method for the invention may also be used when other viral genes or other chemosensitizing genes are employed for gene therapy".

Applicants also respectfully submit that the known art at the time of filing of the priority document fully supports the limitation in claim 8. Applicants submit that it is known to those skilled in the art that the viruses recited in claim 8 of the current application encode for proteins capable of phosphorylating nucleoside analogues. For example, it was known as at the priority date that each of the viruses listed in claim 8 contained a thymidine kinase gene or analogous gene capable of phosphorylating appropriately selected nucleoside analogues (for HSV see Page 1, Line 10-11 of United Kingdom Provisional Application 9421223.0; for human cytomegalovirus see Kulowski, Pharm World Sci. (1994) 16(2):127-138; for varicella zoster virus see De Clerqc, J. Antimicrob. Chemother. (1993) 32 Suppl. A:121-132; and for Epstein-Barr virus see Liu et al., J. Virol. Methods (1992) 40(1):107-118). Therefore, the Applicant submits that there is support for selection of a foreign gene from the viruses recited in Claim 8 present in the priority document, together with the prior art .

In conclusion, Applicants submit that the priority document, the known art at the time of filing, or both, support the limitations contained in Claim 6 and Claim 8. Thus, it is submitted that the findings of the Examiner with respect to claims to priority of claims 1-31 have been traversed and the claims should be awarded the priority date of **October 20, 1994.**

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CLAIM REJECTIONS 35 USC 101

With respect to Claim 32 and the Examiner's rejection based upon a recitation of use without setting forth any steps involved in the process, Applicants submit that new claim 33 clearly contains active, positive steps delineating how the **method** (as opposed to the former previously cited "use") is actually practiced. Applicants cancel claim 32 and respectfully submit that new method claim 33 clearly delineates the invention claimed and sets forth steps involved in the method.

CLAIM REJECTIONS 35 USC 112

The Examiner's rejection of claims 1-16, and 30-32 for lack of enablement for *in vivo* methods of detecting gene transfer in a population of cells using IVDU or IVDU-CDS is traversed for the reasons now following.

Iwashina (Appl. Radiat. Isot. (1990) 41(7): 675-678) discloses that IVDU is catabolized from IVDU to IVU, which is non-toxic and non-selective for HSV TK^+ infections. Though not *efficient* as an imaging agent due to its catabolism *in vivo*, IVDU is *feasible* as an imaging agent, as it would be a suitable substrate for a foreign gene, thymidine kinase (TK), and the detection of the product would be possible using radio-imaging. As taught in Samuel *et al.*, Antimicrob Agents Chemother (1986) 29(2):320:324, IVDU is cleaved to IVU by two enzymes (thymidine phosphorylase and uridine phosphorylase) found in the blood (Samuel *et al.*, page 323, column 1, last paragraph). IVU is not selective for cells with HSV-TK^+ infection (Iwashina (1990), page

675, column 2, line 19-24), yet IVDU is selective for cells expressing HSV-TK (Example 10, current application).

As IVDU is labile *in vivo*, a higher dosage would be necessary, said dosage sufficient to allow entry into cells expressing the selected isolated foreign gene (*i.e.* HSV-TK) and the product arising from the interaction between IVDU and the foreign gene (HSV-TK) being trapped. As disclosed in Figure 3 of Samuel *et al.*, introduction of IVDU into dogs does result in a decrease of IVDU resulting from catabolism to IVU, yet it is a decrease over time and not an immediate and sudden conversion into the non-selective compound IVU. Furthermore, IVU is non-toxic and cleared from the blood (Samuel *et al.*, Figure 4). Therefore increased levels of IVU, arising from increased administration of IVDU (sufficient to allow entry and trapping by HSV-TK containing cells), would be cleared from the blood; leaving trapped labelled product in the HSV-TK containing cells.

Thus, by waiting sufficient time for clearance of the labelled IVU, said time reasonably determinable to one skilled in the art, visualization of the trapped labelled IVDU would be possible. The product arising from the intracellular interaction between IVDU and the foreign expressed gene (HSV-TK) is trapped within the cell and therefore immune to catabolism mediated by the blood enzymes.

Finally, Applicants respectfully submit that neither Iwashina et al (1988) nor Iwashina et al (1990) teach non-specific sequestering of IVU, as submitted by the Examiner on Page 4, paragraph 8. To the contrary IVU is considered to be non-selective for HSV-TK, with trapping

through interaction with HSV-TK only occurring with IVDU, not IVU (Samuel, Page 324, collumn1, first paragraph). Though enzymatic formation of the labelled IVU can mask the signal from trapped labelled IVDU, this is a time and dosage dependent phenomena. Administration of IVDU would simply require higher concentration than other, more *in vivo* stable compounds, which one skilled in the art would realize and be capable of compensating for by dosage administered. The usage of IVDU as a compound applicable for the current invention would not be as efficient as perhaps IVFRU-CDS, but, the Applicant submits, use of IVDU is feasible, particularly when using longer lived isotopes, such as ^{124}I and ^{131}I, as a radiolabel.

Balzarini, Gene Therapy (7/1995) 2(5): 317-322, has shown the efficacy and ability of IVDU-CDS to inhibit TK containing cell lines. The product arising from the interaction between IVDU-CDS and the foreign gene (TK) is being detected in the current invention, with *in vitro* delivery of IVDU-CDS shown in the art (Balzarini) as well as in the disclosed invention (Example 10, Example 12). The measurement of a product arising from interaction between the compound and foreign gene, in the *in vivo* environment, where lability may be an issue, simply requires an increase in administered dose of the compound, sufficient to enable imaging of the extent and location of the product in the patient. Increasing the dosage of IVDU-CDS would be within the knowledge of a skilled person and thus, would not require undue experimentation. Therefore the Applicant respectfully submits that one skilled in the art would reasonably be capable of using the invention as disclosed.

Page 21, lines 1-6 describe a series of compounds, all of formula 1, with substituents described on page 21, lines 9-27. IVFRU is therefore a compound of formula 1, as disclosed in the

specification. Example 14 in the specification describes the introduction of IVFRU and transduced cells into a mouse, with Figures 6 and 7 demonstrating scintigraphic imaging of the mouse, thereby demonstrating the selective "trapping" of the compound. It is submitted that this example demonstrates a reasonable enablement of the invention in an *in vivo* model, namely a live mouse. As stated above, use of IVDU or IVDU-CDS, due to the lability of the compound, would require one to alter the dosage of radiolabelled drug administered, something not requiring undue experimentation and well within the ability of one skilled in the art. The methods taught in the specification enable a person skilled in the art to use the invention commensurate with the scope of the claims in other *in vivo* systems.

It is accordingly respectfully submitted, for the above reasons, that the specification is enabling and does provide adequate support to claim 1-16 and 30-32 (now 33) for *in vivo* methods of detecting gene transfer in a population of cells.

The Examiner further states on Page 6, paragraph 12;

> Claims 1-32 are indefinite because it is unclear what constitutes a 'substantial amount of the labelled compound'. The specification fails to define 'substantial' in this context, and there is no art recognized definition, so one of skill in the art cannot know the metes and bounds of the claims.

The Applicants respectfully submit that there is clear and adequate recital within claim 1 of the present specification to allow one skilled in the art to determine what constitutes " a substantial amount of the labelled compound" for the following reasons:

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Specifically, Applicants state on page 9, lines 4-8, that it is important to wait a period of time after adding the labelled compounds before the step of detecting the labelled product in the cells. Such time period is important in order to minimize the background of labelled compound in the cell in order to enhance or increase the accuracy of the diagnostic method. This is due to the fact that the presence of significant or substantial amounts of labelled compound in the cell may interfere with the detection of the labelled product (see page 9, lines 12-15). Hence, most preferably, it is desirable that all or substantially all labelled compounds have been expelled from the cells while all or substantially all of the labelled product remains within the cells (see page 9, lines 21-24).

The Applicants further states at page 9 line 24-60:

> "In other words the expulsion of the labelled compound and the expulsion of the labelled product do not overlap such that the expulsion of the labelled compound from the cells is complete or substantially complete prior to the commencement of any expulsion of the labelled product from the cells." [emphasis added]

Also at page 10, lines 6- Applicants state:

> "For a substantial amount to be expelled, any remaining labelled compound in the cells is not enough to significantly interfere with the detection of the labelled product and is such that the diagnostic test results achieve the desired degree of accuracy or statistical significance".

The Applicants have been careful to include this functional definition of "substantial" in claim 1 (and thus also all dependent claims 2-31) by inclusion of element d) of the recited method, namely :

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d) waiting a period of time such that a substantial amount of the labelled compound has been expelled from the cells **and** such that a detectable amount of the labelled product remains within the cells.

Therefore the Applicants respectfully submit that the Applicants have functionally recited in claim 1 what constitutes a "substantial amount" in the context of both 1) product to be expelled (ie a substantial amount of the labelled product originally introduced), as well as 2) the quantum which is expelled when a detectable amount of labelled product remains in the cells, and therefore has adequately defined what amount is "substantial". The "substantial amount" is not necessarily one which can be defined in terms of precise quantum, because it may depend on a number of factors, such as the amount of labelled compound originally provided, and the degree to which a particular selected isolated gene may convert same into labelled product. It is submitted that a person skilled in the art would necessarily know the metes and bounds of the "substantial" amount due because a skilled person would recognize that in order to accurately detect resulting labelled product it would be desirable to reduce the background amount of labelled compound in the cells, *i.e.* the labelled compound which hasn't been converted to labelled product.

CLAIM REJECTIONS 35 USC 102

Cited References

Dougan (United States Patent 5,248,771) teaches a process of producing IVaraU, a nucleoside analogue, with anti-viral effects and the potential for imaging HSV infection. Dougan further

discloses the uptake of IVaraU in rabbit brain infected with HSV1 compared to non-infected areas of the rabbit brain (Column 15, lines 5-8). The administered compound is visualised and serves as a means of detecting HSV1 infection through ^{125}I labelling of the IVaraU.

Gill (Antimicrob. Agents and Chmother. (1984) 25(4): 476-478) proposes a non-invasive, herpes specific diagnostic test for particular use in the diagnosis of herpes simplex encephalitis. The proposed test involves the administration of a radiolabelled antiviral compound, particularly, [^{131}I]IVDU. To study the potential diagnostic capabilities of the proposed test, a population of cultured cells was infected by an infecting dose of HSV type 1 infecting agent known to cause viral encephalitis. The radiolabelled compound was then administered to the cultured cell population and the cellular uptake of the radiolabelled compound was then quantified. In this respect, it was found that the radiolabelled compound was selectively trapped within HSV infected cells having virally specified thymidine kinase. Accordingly, a quantitative evaluation could be performed on the uptake of the radiolabelled compound by the HSV infected cell population.

Iwashina (Drug Design and Delivery (1988) 2(4): 309-321) teaches the synthesis of IVFRU and its ^{125}I and ^{131}I derivatives. Furthermore, Iwashina compares the sequestering of IVFRU and its derivatives in HSV-1 infected rabbit kidney cells. The cellular uptake of the radiolabelled compound was quantified in cells infected with HSV-1 TK^+, TK^-, and mock infected rabbit kidney cells *in vitro*.

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Applicants respectfully submit that Blasberg (United States Patent 5,703,056) is not relevant prior art for the purposes of 35 U.S.C. 102(e) due to the earlier **October 20, 1994** priority date held by the Applicants.

Each of the cited references, namely, Dougan, Gill and Iwashina, are distinguishable from the present invention in that they all deal with an infection process. None of the references teach the step of selecting a foreign gene which has been isolated from a cell or virus and transferring said isolated foreign gene into a mammalian cell population. Further, these references deal with the monitoring or detection of the incorporation or uptake of the labelled compound or radiolabelled analogue into the infected cell population.

In contrast, the Applicants' invention as claimed does not measure or detect the labelled analogue, being the "labelled compound" of the Applicants' invention. Rather, the Applicants' invention is directed at detecting the "labelled product". More particularly, the labelled compound is administered to the cell population, wherein the labelled compound interacts selectively with a protein expressed by the foreign gene to produce a labelled product. In this regard, the labelled compound is particularly selected such that the labelled product resulting from the selective interaction becomes trapped within those of the cells in which the protein has been expressed by the foreign gene. Accordingly, the Applicants' invention may determine the extent and location of the protein expressed by the foreign gene by detecting the labelled product, in a preferably non-invasive manner such as nuclear medicine imaging.

A clear distinction exists between determining the presence of the labelled compound (as in the prior art) and determining the presence (particularly the extent and location) of the expressed protein by detecting the labelled product. The need for this distinction and the need for determining the extent and location of the protein through detection of the labelled product is discussed in the specification as follows.

Page 3, lines 17-29 of the specification discusses potential limitations of various gene therapy techniques. One limitation is that "gene transfer does not necessarily mean that the gene is actually expressed to give the active protein throughout the target tissue." This limitation is further discussed at Page 13, line 28 to Page 14, Line 12:

> "Transfer of the foreign gene into the cells does not necessarily mean that every transferred foreign gene is actively expressing the protein into the cell in which that specific foreign gene is located. Some foreign genes may be actively expressing the protein, while others may be dormant. As a result, the labelled product is produced only within those cells in which the protein has actually been expressed by the foreign gene. The labelled product may then be detected in order to monitor the transfer of the foreign gene in the cells.
>
> The labelled compound is selected to interact selectively with, or be acted on by, the specific protein expressed by the foreign gene in order to produce a labelled product which is trapped and thus localized within and which does not readily escape from, the cells in which the protein has been expressed. Thus a

> preferential accumulation or localization or a selective metabolic trapping of the labelled product occurs in the protein expressing cells, as compared to cells which either do not include the foreign gene or which include a dormant foreign gene. This selective trapping permits the specific detection of those cells which both include the foreign gene and in which the specific protein has been expressed."

Accordingly, the Applicants' invention provides for an interventional process in which a foreign gene is specifically selected, which is isolated from a cell or virus and transferred into a target cell population. The foreign gene and a labelled compound administered to the cell population are both particularly selected such that the labelled compound selectively interacts with a protein expressed by the selected, isolated foreign gene; to produce a labelled product which becomes trapped within those cells in which the protein has been expressed. Further, the Applicants' invention then allows the determination of the extent and location of the protein throughout the cell population by detecting the labelled product. In other words, the Applicants' invention allows the presence of expressing foreign gene within the cell population to be monitored.

As a result, the Applicants' invention as claimed differs from the cited prior art in at least two primary respects. First, all of the references deal with the monitoring or detection of the incorporation of the labelled compound or radiolabelled analogue into the cell population. This measurement or method of monitoring does not indicate or determine the extent or location of the protein expressed by the foreign gene. Thus, there is no indication of whether or not any transferred foreign genes in the cell population are actively expressing the protein or whether

they are dormant. In fact, none of the references describe, disclose or teach in any manner the detection, preferably the non-invasive detection in a living animal or patient, of a labelled product resulting from the selective interaction of a protein expressed by a selected foreign gene and the selected labelled compound.

Second, the references particularly deal with an infection process. During a viral infection, all of the genes of the particular virus, such as HSV, are transferred into the host cell population and all of the genes may be involved in active protein expression. There is no selection of a particular foreign gene, isolation of the selected foreign gene from a cell or virus, or transfer of the isolated foreign gene into the cell population.

Addressing more specifically the Examiner's rejection based on Dougan and Iwashina, on page 8, paragraph 16 of the Office Action the Examiner states that "[b]ecause herpes simplex virus must be produced in cells and isolated for use, the method of Dougan fairly suggests the isolation from cells of the Herpes virus and the Herpes thymidine kinase gene". Further, on page 9, paragraph 21, the Examiner states "[s]uch viruses, and their genes, are considered to be isolated from a cell prior to use, because viruses must be produced by infection and subsequent isolation from cells". These statements are used to support the Examiner's contention that the Applicants' invention is anticipated by Dougan and Iwashina respectively. The Applicants submit this to not be accurate.

Neither Dougan nor Iwashina teach the step of **selection** of a foreign gene which is **isolated** from a cell or virus and **transferred** into a cell. While the viral replication cycle does include the

introduction of foreign genes (viral) into the host cell, it necessarily involves the introduction of the entire viral genome. There is no isolation, directed by a conscious mind, of a particular foreign (*i.e.* in this case viral) gene. Thus, there is a wholesale transfer of the entire sequence of the virus, by natural infection and replication, which brings with it the entirety of the viral genome potentially leading to an active infection.

The Applicants' invention teaches the **isolation** of a foreign gene. The cited references deal with the infection process (and detection thereof), while the Applicants teach a method of detecting the presence of a specifically **selected** and **isolated** foreign gene and detection of the **transfer** of the foreign gene to a cell based on the foreign gene product's interaction with a labelled compound to produce a labelled product. It is the entirety of infection detected in the cited references, and the "isolation" of HSV required for its "use" as proposed by the Examiner is in reality the detection of infection, not the selection, isolation and transfer of a foreign gene into a mammalian cell. Furthermore the genes transferred by native HSV infection are limited to only those genes naturally present in the viral genome, while the present invention addresses the detection of transfer of any selected, isolated foreign gene.

Thus, in summary, none of the references cited by the Examiner discuss, teach or suggest the steps of selecting a foreign gene, namely one which has been isolated from a cell or virus; and transferred into a cell population, and thereafter administering a particular selected labelled compound which will interact selectively with a protein expressed by said particular selected gene to produce a labelled product, and thereafter determining the extent and location of a protein expressed by such foreign gene by detecting the presence of such labelled product.

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Again, to emphasize, the labelled compound is selected to interact selectively with the protein expressed by the foreign gene to produce the labelled product which is trapped within those of the cells in which the protein has been expressed by the foreign gene.

CLAIM REJECTIONS 35 USC 103

The Examiner rejects claim 15 of the current application under 35 USC 103(a) as being unpatentable over that taught by Gill in light of Dougan. Applicants submits that the Examiner has failed to prove a prima facie case of obviousness for the reasons now following and for the reasons given above in respect to the Examiner's rejection based on anticipation by Gill.

To summarize, Gill teaches the use of radiolabelled compounds to diagnose HSV infections by detection of the labelled analogue or "labelled compound", while the present invention is directed at detecting the "labelled product". Furthermore, Gill teaches the use of certain compounds to diagnose infection by HSV in a cell population, while the current invention is directed at detecting the expression of a selected, isolated foreign protein, transferred into and expressed in a cell population. As the method taught by Gill relates to diagnosis of HSV infection and not the presence of an isolated foreign protein, it is submitted by the Applicants that use of ^{123}I or ^{131}I does not render the present invention obvious with respect to Gill in light of Dougan.

The Examiner rejects claims 14-25, 28 and 29 of the current application under 35 USC 103(a) as being unpatentable over that taught by Dougan in light of Balzarini. The Applicants respectfully

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submit that for the reasons submitted with respect to the Examiner's statement that the invention is anticipated by Dougan, contained herein, claims 14-25, 28 and 29 are not rendered obvious and thus the Examiner has failed to make out a *prima facie* case for obviousness.

To summarize, Dougan teaches a process of producing IVaraU, a nucleoside analogue, with anti-viral effects and the potential for imaging HSV infection by detection of the of the labelled analogue or "labelled compound", while the present invention is directed at detecting the "labelled product". Furthermore, Dougan teaches the use of certain compounds to diagnose infection by HSV in a cell population, specifically rabbit brain tissue; while the current invention is directed at detecting the expression of a selected, isolated foreign protein, transferred into and expressed in a cell population. As Dougan's method of monitoring HSV infection does not anticipate imaging or monitoring the transfer and expression of a selected, isolated foreign gene as submitted by the Applicants herein, the Applicants' use of the compounds as taught in Balzarini can not render the invention obvious in light of Dougan.

In contrast, the Applicants' invention as claimed does not measure or detect the labelled analogue, being the "labelled compound", of the cited references. Rather, the Applicants' invention is directed at detecting the "labelled product". More particularly, the labelled compound is administered to the cell population, wherein the selected labelled compound interacts selectively with a protein expressed by the selected foreign gene to produce a labelled

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product.

Favourable consideration of this application, including new claim 33, is earnestly solicited, in view of the revisions/amendments to the claims and the submissions and comments made above, with a view to allowance of the claims as now contained in this application.

Respectfully Submitted,

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Agents for the Applicants